Stem Cells .. Basics And Applications

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May 18, 2006

I- Introduction

A- Definition

Stem cells are cells that divide to form one daughter cell that goes on to differentiate, and one daughter cell that retains its stem-cell properties. All stem cells regardless of their source have three general properties : they are unspecialized, they are capable of dividing and renewing themselves for long periods, and under certain physiologic or experimental conditions, they can be induced to become cells with special functions, i.e. they can give rise to specialized cell types.

B- Importance of stem cells

Stem cells are important for living organisms for many reasons. In the 3- to 5-day-old embryo, called a **blastocyst**, stem cells in developing tissues give rise to the multiple specialized cell types that make up the heart, lung, skin, and other tissues. In some adult tissues, such as bone marrow, muscle, and brain, discrete populations of adult stem cells generate replacements for cells that are lost through normal wear and tear, injury, or disease.

C- Sources of stem cells

Ways to obtain or derive stem cells from early mouse embryos have been discovered more than 20 years ago. Many years of detailed study of the biology of mouse stem cells led to the discovery, in 1998, of how to isolate stem cells from human embryos and grow the cells in the laboratory. These are called **human embryonic stem cells (hESC).** The embryos used in these studies were created for infertility purposes through **in vitro fertilization** or pre-implantation genetic diagnosis. Research on stem cells involves studying and manipulating two kinds of these cells from animals and humans : **embryonic stem cells** and **adult stem cells**, which have different functions and characteristics.

Human embryonic stem cells are produced from chromosomally euploid, aneuploid, and mutant human embryos. These hESC lines are an important resource for functional genomics, drug screening, and, perhaps eventually, cell and gene therapy.

The methods for deriving hESCs are well established and repeatable and are relatively successful with a ratio of 1 : 10 to 1 : 2 new hESC lines produced from 4 to 8 days old morula and blastocysts and from isolated inner cell mass cell clusters of human blastocysts.
The hESCs can be formed and maintained on human somatic cells in humanized serum-free culture conditions and for several passages in cell-free culture systems. The hESCs can be transfected with DNA constructs. They may be grown indefinitely in vitro while maintaining their original karyotype and epigenetic status, but this needs to be confirmed from time to time in long-term cultures.

hESCs spontaneously differentiate in the absence of the appropriate cell feeder layer, when overgrown in culture and when isolated from the ESC colony.

All three major embryonic lineages are produced in differentiating flat attachment cultures and unattached embryoid bodies. Cell progenitors of interest can be identified by markers, expression of reporter genes, and characteristic morphology, and the cells thereafter enriched for progenitor types and further culture to more mature cell types.

D- Prospects of stem cells

Research on stem cells aims at disclosing how an organism develops from a single cell and how healthy cells replace damaged cells in adult organisms. This promising area of science is also leading scientists to investigate the possibility of cell-based therapies to treat disease, which is often referred to as **regenerative or reparative medicine**. It has been hypothesized that stem cells may, at some point in the future, become the basis for treating diseases such as Parkinson's disease, diabetes, and heart disease. As we learn more about stem cells, it may become possible to use the cells not just in **cell-based therapies**, but also for screening new drugs and toxins and understanding birth defects.

E- Spectrum of stem cell research

In order to develop stem cells-based treatments and screening tools, many aspects of stem cell's biology have to be disclosed. These include understanding the fundamental properties of stem cells that relate to their **long-term self-renewal, their dormancy, and their selective potentials of differentiation.** In this regard, three basic qustions arise :
1- Why can **embryonic stem cells** proliferate for a year or more in the laboratory without differentiating, while most **adult stem cells** cannot ?.
2- What are the genetic factors in living organisms that normally regulate stem cell **proliferation** and self-renewal ?.
3- What are the genetic mechanisms that control and direct the **signaling** networks underlying differentiation and specialization of stem cells.
Discovering the answers to these questions may make it possible to understand how cell proliferation is regulated during normal embryonic development or during the abnormal **cell**

division that leads to cancer. Importantly, such information would enable scientists to grow embryonic and adult stem cells more efficiently in the laboratory.

II- Stem Cells In Early Development

A- What are embryonic stem cells ?

Embryonic stem cells, as their name suggests, are derived from embryos. Specifically, embryonic stem cells are derived from embryos that develop from eggs that have been fertilized **in vitro**—in an **in vitro fertilization** clinic—and then donated for research purposes with informed consent of the donors. They are not derived from eggs fertilized in a woman's body. The **embryos** from which **human embryonic stem cells** are derived are typically four or five days old and are a hollow microscopic ball of cells called the **blastocyst**. The blastocyst includes three structures: the **trophoblast**, which is the layer of cells that surrounds the blastocyst; the **blastocoel**, which is the hollow cavity inside the blastocyst; and the **inner cell mass**, which is a group of approximately 30 cells at one end of the blastocoel.

Fetal development begins with **totipotent** cells which include the fertilized egg (The zygote) and the first 4 or so cells produced by its cleavage (as shown by the ability of mammals to produce identical twins, triplets, etc.). In mammals, totipotent cells have the potential to become any type in the adult body and / or any cell of the extraembryonic membranes (e.g., placenta). In mammals, the expression **totipotent stem cells** is a misnomer because these cells cannot make more of themselves.

True fetal stem cells comprise two cell types :

A- Pluripotent stem cells

These are true stem cells, with the potential to make any differentiated cell in the body, but cannot contribute to making the extraembryonic membranes (which are derived from the trophoblast). They include three types of cells :

1- Embryonic Stem (ES) Cells.

These can be isolated from the **inner cell mass** (ICM) of the blastocyst — the stage of embryonic development when implantation occurs. For humans, excess embryos produced during in vitro fertilization (IVF) procedures are used. Harvesting ES cells from human blastocysts is controversial because it destroys the embryo, which could have been implanted to produce another baby (but often was simply going to be discarded).

2- Embryonic Germ (EG) Cells.

These can be isolated from the precursor to the gonads in aborted fetuses.

3- Embryonic Carcinoma (EC) Cells.

These can be isolated from teratocarcinomas, a tumor that occasionally occurs in a gonad of a fetus. Unlike the other two, they are usually aneuploid.

All three of these types of pluripotent stem cells can only be isolated from embryonic or fetal tissue and can be grown in culture, but only with special methods to prevent them from differentiating.

B- Multipotent stem cells.

These are true stem cells but can only differentiate into a limited number of types. For example, the bone marrow contains multipotent stem cells that give rise to all the cells of the blood but not to other types of cells.

Multipotent stem cells are found in adult animals; perhaps most organs in the body (e.g., brain, liver) contain them where they can replace dead or damaged cells. These **adult stem cells** may also be the cells that - when one accumulates sufficient mutations - produce a clone of cancer cells.

III- Biological Characteristics Of Stem Cells

Stem cells are unspecialized. One of the fundamental properties of a stem cell is that it does not have any tissue-specific structures that allow it to perform specialized functions. A stem cell cannot work with its neighbors to pump blood through the body (like a heart muscle cell); it cannot carry molecules of oxygen through the bloodstream (like a red blood cell); and it cannot fire electrochemical **signals** to other cells that allow the body to move or speak (like a nerve cell). However, unspecialized stem cells can give rise to specialized cells, including heart muscle cells, blood cells, or nerve cells.

Stem cells are capable of dividing and renewing themselves for long periods. Unlike muscle cells, blood cells, or nerve cells—which do not normally replicate themselves—stem cells may replicate many times. When cells replicate themselves many times over it is called proliferation. A starting population of stem cells that proliferates for many months in the laboratory can yield millions of cells. If the resulting cells continue to be unspecialized, like the parent stem cells, the cells are said to be capable of long-term self-renewal.

The specific factors and conditions that allow stem cells to remain unspecialized are of great interest to scientists. It has taken scientists many years of trial and error to learn to grow stem cells in the laboratory without them spontaneously differentiating into specific cell types. For example, it took 20 years to learn how to grow **human embryonic stem cells** in the laboratory following the development of conditions for growing mouse stem cells. Therefore, an important area of research is understanding the signals in a mature organism that cause a stem cell population to proliferate and remain unspecialized until the cells are needed for repair of a specific tissue. Such information is critical for scientists to be able to grow large numbers of unspecialized stem cells in the laboratory for further experimentation.

Stem cells can give rise to specialized cells. When unspecialized stem cells give rise to specialized cells, the process is called **differentiation**. Scientists are just beginning to understand the signals inside and outside cells that trigger stem cell differentiation. The internal signals are controlled by a cell's **genes**, which are interspersed across long strands of DNA, and carry coded instructions for all the structures and functions of a cell. The external signals for cell differentiation include chemicals secreted by other cells, physical contact with neighboring cells, and certain molecules in the **microenvironment**.

Therefore, many questions about stem cell differentiation remain. For example, are the internal and external signals for cell differentiation similar for all kinds of stem cells ? Can specific sets of signals be identified that promote differentiation into specific cell types ? Addressing these questions is critical because the answers may lead scientists to find new ways of controlling stem cell differentiation in the laboratory, thereby growing cells or tissues that can be used for specific purposes including **cell-based therapies**.

Adult stem cells typically generate the cell types of the tissue in which they reside. A blood-forming adult stem cell in the bone marrow, for example, normally gives rise to the many types of blood cells such as red blood cells, white blood cells and platelets. Until recently, it had been thought that a blood-forming cell in the bone marrow which is called a **hematopoietic stem cell** could not give rise to the cells of a very different tissue, such as nerve cells in the brain. However, a number of experiments over the last several years have raised the possibility that stem cells from one tissue may be able to give rise to cell types of a completely different tissue, a phenomenon known as **plasticity**. Examples of such plasticity include blood cells becoming **neurons**, liver cells that can be made to produce insulin, and hematopoietic stem cells that can develop into heart muscle. Therefore, exploring the possibility of using adult stem cells for cell-based therapies has become a very active area of investigation by researchers.

IV- Using Stem Cells for Human Therapy

Many diseases arise from damage to differentiated cells. Examples include :
1- Insulin-dependent diabetes mellitus (IDDM) where the beta cells of the pancreas have been destroyed by an autoimmune attack.
2- Parkinson's disease; where dopamine-secreting cells of the brain have been destroyed.
3- Spinal cord injuries leading to paralysis of the skeletal muscles; [View]
4- Ischemic stroke where a blood clot in the brain has caused neurons to die from oxygen starvation.
5- **Multiple sclerosis** with its loss of myelin sheaths around axons.
6- Blindness caused by damage to the cornea.

The great developmental potential of stem cells has created intense research into enlisting them to aid in replacing the lost cells of such disorders. While some success has been achieved with laboratory animals, not much has yet been achieved with humans. One exception is the culturing of human epithelial stem cells and using their differentiated progeny to replace a damaged cornea. This works best when the stem cells are from the patient (e.g. from the other eye). Corneal cells from another person (an allograft) are always at risk of rejection by the recipient's immune system.

A- The Problems

One major problem that must be solved before human stem cell therapy becomes a reality is the threat of rejection of the transplanted cells by the host's immune system (if the stem cells are allografts; that is, come from a genetically-different individual).

B- The Solutions

One way to avoid the problem of rejection is to use stem cells that are genetically identical to the host. This is already possible in the rare situations when the patient has healthy stem cells in an undamaged part of the body (like the stem cells being used to replace damaged corneas).
But even where no "autologous" stems cells are available, there may be a solution : using somatic-cell nuclear transfer (but with no goal of attempting to implant the resulting blastocyst in a uterus).

V. Practical Aspects Of Embryonic Stem Cell Research

A. How are embryonic stem cells grown in the laboratory ?

Growing cells in the laboratory is known as **cell culture**. Human embryonic stem cells are isolated by transferring the **inner cell mass** into a plastic laboratory culture dish that contains a nutrient broth known as **culture medium**. The cells divide and spread over the surface of the dish. The inner surface of the culture dish is typically coated with mouse embryonic skin cells that have been treated so they will not divide. This coating layer of cells is called a **feeder layer**. The reason for having the mouse cells in the bottom of the culture dish is to give the inner cell mass cells a sticky surface to which they can attach. Also, the feeder cells release nutrients into the culture medium. Recently, scientists have begun to devise ways of growing embryonic stem cells without the mouse feeder cells. This is a significant scientific advancement because of the risk that viruses or other macromolecules in the mouse cells may be transmitted to the human cells.

Over the course of several days, the cells of the inner cell mass proliferate and begin to crowd the culture dish. When this occurs, they are removed gently and plated into several fresh culture dishes. The process of replating the cells is repeated many times and for many months, and is called **subculturing**. Each cycle of subculturing the cells is referred to as a **passage**. After six months or more, the original 30 cells of the inner cell mass yield millions of embryonic stem cells. Embryonic stem cells that have proliferated in cell culture for six or more months without differentiating, are **pluripotent**, and appear genetically normal are referred to as an **embryonic stem cell line**.

Once cell lines are established, or even before that stage, batches of them can be frozen and shipped to other laboratories for further culture and experimentation.

B. What laboratory tests are used to identify embryonic stem cells ?

At various points during the process of generating embryonic stem cell lines, scientists test the cells to see whether they exhibit the fundamental properties that make them embryonic stem cells. This process is called characterization.

As yet, scientists who study human embryonic stem cells have not agreed on a standard battery of tests that measure the cells' fundamental properties. Also, scientists acknowledge that many of the tests they do use may not be good indicators of the cells' most important biological properties and functions. Nevertheless, laboratories that grow human embryonic stem cell lines use several kinds of tests. These tests include :

1- Growing and subculturing the stem cells for many months. This ensures that the cells are capable of long-term self-renewal. Scientists inspect the cultures through a microscope to see that the cells look healthy and remain **undifferentiated**.

2- Using specific techniques to determine the presence of **surface markers** that are found only on undifferentiated cells. Another important test is for the presence of a protein called Oct-4, which undifferentiated cells typically make. Oct-4 is a transcription factor, meaning that it helps turn **genes** on and off at the right time, which is an important part of the processes of cell **differentiation** and embryonic development.

3- Examining the chromosomes under a microscope. This is a method to assess whether the chromosomes are damaged or if the number of chromosomes has changed. It does not detect genetic mutations in the cells.

4- Determining whether the cells can be subcultured after freezing, thawing, and replating.

5- Testing whether the human embryonic stem cells are pluripotent by 1) allowing the cells to differentiate spontaneously in cell culture; 2) manipulating the cells so they will differentiate to

form specific cell types; or 3) injecting the cells into an immunosuppressed mouse to test for the formation of a benign tumor called a **teratoma**. Teratomas typically contain a mixture of many differentiated or partly differentiated cell types, an indication that the embryonic stem cells are capable of differentiating into multiple cell types.

C. How are embryonic stem cells stimulated to differentiate ?

As long as the embryonic stem cells in culture are grown under certain conditions, they can remain undifferentiated (unspecialized). But if cells are allowed to clump together to form **embryoid bodies**, they begin to differentiate spontaneously. They can form muscle cells, nerve cells, and many other cell types. Although spontaneous differentiation is a good indication that a culture of embryonic stem cells is healthy, it is not an efficient way to produce cultures of specific cell types.

So, to generate cultures of specific types of differentiated cells- heart muscle cells, blood cells, or nerve cells, for example - scientists try to control the differentiation of embryonic stem cells. They change the chemical composition of the culture medium, alter the surface of the culture dish, or modify the cells by inserting specific genes. Through years of experimentation scientists have established some basic protocols or "recipes" for the **directed differentiation** of embryonic stem cells into some specific cell types.

If scientists can reliably direct the differentiation of embryonic stem cells into specific cell types, they may be able to use the resulting, differentiated cells to treat certain diseases at some point in the future. Diseases that might be treated by transplanting cells generated from human embryonic stem cells include Parkinson's disease, diabetes, traumatic spinal cord injury, Purkinje cell degeneration, Duchenne's muscular dystrophy, heart disease, and vision and hearing loss.

VI- Adult Stem Cells

A- Introduction

An adult stem cell is an **undifferentiated** cell found among differentiated cells in a tissue or organ, can renew itself, and can differentiate to yield the major specialized cell types of the tissue or organ.

B- Function(s)

The primary roles of **adult stem cells** in a living organism are to maintain and repair the tissue in which they are found. Some scientists now use the term **somatic stem cell** instead of adult stem cell. Unlike **embryonic stem cells**, which are defined by their origin (the **inner cell mass** of the **blastocyst**), the origin of adult stem cells in mature tissues is unknown.

C- Sources

Adult stem cells have been identified in many organs and tissues. One important point to understand about adult stem cells is that there are a very small number of stem cells in each tissue. Stem cells are thought to reside in a specific area of each tissue where they may remain quiescent (non-dividing) for many years until they are activated by disease or tissue injury. The adult tissues reported to contain stem cells include brain, bone marrow, peripheral blood, blood vessels, skeletal muscle, skin and liver. However, adult stem cells have been found in many more tissues than they once thought possible.

D- Prospects of adult stem cells

The wide distribution of adult stem cells in nearly all body organs has led scientists to ask whether adult stem cells could be used for transplants. In fact, adult blood forming stem cells from bone marrow have been used in transplants for 30 years. Certain kinds of adult stem cells seem to have the ability to differentiate into a number of different cell types, given the right conditions. If this differentiation of adult stem cells can be controlled in the laboratory, these cells may become the basis of therapies for many serious common diseases.

The history of research on adult stem cells began about 40 years ago. In the 1960s, researchers discovered that the bone marrow contains at least two kinds of stem cells. One population, called **hematopoietic stem cells**, forms all the types of blood cells in the body. A second population, called **bone marrow stromal cells**, was discovered a few years later. **Stromal cells** are a mixed cell population that generates bone, cartilage, fat, and fibrous connective tissue.

Also in the 1960s, scientists who were studying rats discovered two regions of the brain that contained dividing cells, which become nerve cells. Despite these reports, most scientists believed that new nerve cells could not be generated in the adult brain. It was not until the 1990s that scientists agreed that the adult brain does contain stem cells that are able to generate the brain's three major cell types : **astrocytes** and **oligodendrocytes**, which are non-neuronal cells, and **neurons**, or nerve cells.

Scientists in many laboratories are trying to find ways to grow adult stem cells in **cell culture** and manipulate them to generate specific cell types so they can be used to treat injury or disease. Some examples of potential treatments include replacing the dopamine-producing cells in the brains of Parkinson's patients, developing insulin-producing cells for type I diabetes and repairing damaged heart muscle following a heart attack with cardiac muscle cells.

E. Criteria for identifying adult stem cells

Scientists do not agree on the criteria that should be used to identify and test adult stem cells. However, they often use one or more of the following three methods : (1) labeling the cells in a living tissue with molecular markers and then determining the specialized cell types they generate.
(2) removing the cells from a living animal, labeling them in cell culture, and transplanting them back into another animal to determine whether the cells repopulate their tissue of origin.
(3) isolating the cells, growing them in cell culture, and manipulating them, often by adding growth factors or introducing new **genes**, to determine what differentiated cells types they can become.

Also, a single adult stem cell should be able to generate a line of genetically identical cells - known as a **clone** - which then gives rise to all the appropriate differentiated cell types of the tissue. Scientists tend to show either that a stem cell can give rise to a clone of cells in cell culture, or that a purified population of candidate stem cells can repopulate the tissue after transplant into an animal. Recently, by infecting adult stem cells with a virus that gives a unique identifier to each individual cell, scientists have been able to demonstrate that individual adult stem cell clones have the ability to repopulate injured tissues in a living animal.

F. Differentiation of adult stem cell

Adult stem cells occur in many tissues and they enter normal **differentiation** pathways to form the specialized cell types of the tissue in which they reside. Adult stem cells may also exhibit the ability to form specialized cell types of other tissues, which is known as **transdifferentiation** or **plasticity**.

Normal differentiation pathways of adult stem cells

In a living animal, adult stem cells can divide for a long period and can give rise to mature cell types that have characteristic shapes and specialized structures and functions of a particular tissue. The following are examples of differentiation pathways of adult stem cells.

Hematopoietic stem cells give rise to all the types of blood cells: red blood cells, B lymphocytes, T lymphocytes, natural killer cells, neutrophils, basophils, eosinophils, monocytes, macrophages, and platelets.

Bone marrow stromal cells (**mesenchymal stem cells**) give rise to a variety of cell types: bone cells (osteocytes), cartilage cells (chondrocytes), fat cells (adipocytes), and other kinds of connective tissue cells such as those in tendons.

Neural stem cells in the brain give rise to its three major cell types: nerve cells (neurons) and two categories of non-neuronal cells—astrocytes and oligodendrocytes.

Epithelial stem cells in the lining of the digestive tract occur in deep crypts and give rise to several cell types: absorptive cells, goblet cells, Paneth cells, and enteroendocrine cells.

Skin stem cells occur in the basal layer of the epidermis and at the base of hair follicles. The epidermal stem cells give rise to keratinocytes, which migrate to the surface of the skin and form a protective layer. The follicular stem cells can give rise to both the hair follicle and to the epidermis.

Adult stem cell plasticity and trans-differentiation.

A number of experiments have suggested that certain adult stem cell types are **pluripotent**. This ability to differentiate into multiple cell types is called plasticity or transdifferentiation. The following list offers examples of adult stem cell plasticity that have been reported during the past few years.

Hematopoietic stem cells may differentiate into three major types of brain cells (neurons, oligodendrocytes, and astrocytes); skeletal muscle cells; cardiac muscle cells; and liver cells.

Bone marrow stromal cells may differentiate into cardiac muscle cells and skeletal muscle cells.

Brain stem cells may differentiate into blood cells and skeletal muscle cells.

Current research is aimed at determining the mechanisms that underlie adult stem cell plasticity. If such mechanisms can be identified and controlled, existing stem cells from a healthy tissue might be induced to repopulate and repair a diseased tissue.

G. Key questions about adult stem cells

Many important questions about adult stem cells remain to be answered. They include :

1- How many kinds of adult stem cells exist, and in which tissues do they exist ?

2- What are the sources of adult stem cells in the body ? Are they "leftover" embryonic stem cells, or do they arise in some other way ? Why do they remain in an undifferentiated state when all the cells around them have differentiated ?

3- Do adult stem cells normally exhibit plasticity or do they only transdifferentiate when scientists manipulate them experimentally ? What are the **signals** that regulate the **proliferation** and differentiation of stem cells that demonstrate plasticity ?.
4- Is it possible to manipulate adult stem cells to enhance their proliferation so that sufficient tissue for transplants can be produced ?.
5- Does a single type of stem cell exist—possibly in the bone marrow or circulating in the blood—that can generate the cells of any organ or tissue ?.
6- What are the factors that stimulate stem cells to relocate to sites of injury or damage ?.

H. The similarities and differences between embryonic and adult stem cells

Human embryonic and adult stem cells each have advantages and disadvantages regarding potential use for cell-based regenerative therapies. Of course, adult and embryonic stem cells differ in the number and type of differentiated cells types they can become. Embryonic stem cells can become all cell types of the body because they are pluripotent. Adult stem cells are generally limited to differentiating into different cell types of their tissue of origin. However, some evidence suggests that adult stem cell plasticity may exist, increasing the number of cell types a given adult stem cell can become.

Large numbers of embryonic stem cells can be relatively easily grown in culture, while adult stem cells are rare in mature tissues and methods for expanding their numbers in cell culture have not yet been worked out. This is an important distinction, as large numbers of cells are needed for stem cell replacement therapies.

A potential advantage of using stem cells from an adult is that the patient's own cells could be expanded in culture and then reintroduced into the patient. The use of the patient's own adult stem cells would mean that the cells would not be rejected by the immune system. This represents a significant advantage as immune rejection is a difficult problem that can only be circumvented with immunosuppressive drugs.

Embryonic stem cells from a donor introduced into a patient could cause transplant rejection. However, whether the recipient would reject donor embryonic stem cells has not been determined in human experiments.

VII. The Potential Uses Of Human Stem Cells

There are many ways in which human stem cells can be used in basic research and in clinical research. However, there are many technical hurdles between the promise of stem cells and the realization of these uses, which will only be overcome by continued intensive stem cell research.

Studies of **human embryonic stem cells** may yield information about the complex events that occur during human development. A primary goal of this work is to identify how **undifferentiated** stem cells become differentiated. Scientists know that turning **genes** on and off is central to this process. Some of the most serious medical conditions, such as cancer and birth defects, are due to abnormal **cell division** and **differentiation**. A better understanding of the genetic and molecular controls of these processes may yield information about how such diseases arise and suggest new strategies for therapy. A significant hurdle to this use and most uses of stem cells is that scientists

do not yet fully understand the **signals** that turn specific genes on and off to influence the differentiation of the stem cell.

Human stem cells could also be used to test new drugs. For example, new medications could be tested for safety on differentiated cells generated from human **pluripotent** cell lines. Other kinds of cell lines are already used in this way. Cancer cell lines, for example, are used to screen potential anti-tumor drugs. But, the availability of pluripotent stem cells would allow drug testing in a wider range of cell types. However, to screen drugs effectively, the conditions must be identical when comparing different drugs. Therefore, scientists will have to be able to precisely control the differentiation of stem cells into the specific cell type on which drugs will be tested. Current knowledge of the signals controlling differentiation fall well short of being able to mimic these conditions precisely to consistently have identical differentiated cells for each drug being tested.

Perhaps the most important potential application of human stem cells is the generation of cells and tissues that could be used for **cell-based therapies**. Today, donated organs and tissues are often used to replace ailing or destroyed tissue, but the need for transplantable tissues and organs far outweighs the available supply. Stem cells, directed to differentiate into specific cell types, offer the possibility of a renewable source of replacement cells and tissues to treat diseases including Parkinson's and Alzheimer's diseases, spinal cord injury, stroke, burns, heart disease, diabetes, osteoarthritis, and rheumatoid arthritis.

For example, it may become possible to generate healthy heart muscle cells in the laboratory and then transplant those cells into patients with chronic heart disease. Preliminary research in mice and other animals indicates that bone marrow stem cells, transplanted into a damaged heart, can generate heart muscle cells and successfully repopulate the heart tissue. Other recent studies in **cell culture** systems indicate that it may be possible to direct the **differentiation** of embryonic stem cells or adult bone marrow cells into heart muscle cells.

In people who suffer from type I diabetes, the cells of the pancreas that normally produce insulin are destroyed by the patient's own immune system. New studies indicate that it may be possible to direct the differentiation of human embryonic stem cells in cell culture to form insulin-producing cells that eventually could be used in transplantation therapy for diabetics.

To realize the promise of novel cell-based therapies for such pervasive and debilitating diseases, scientists must be able to easily and reproducibly manipulate stem cells so that they possess the necessary characteristics for successful differentiation, transplantation and engraftment. The following is a list of steps in successful cell-based treatments that scientists will have to learn to precisely control to bring such treatments to the clinic.

To be useful for transplant purposes, stem cells must be reproducibly made to :

1- Proliferate extensively and generate sufficient quantities of tissue.
2- Differentiate into the desired cell type(s).
3- Survive in the recipient after transplant.
4- Integrate into the surrounding tissue after transplant.

5- Function appropriately for the duration of the recipient's life.
6- Avoid harming the recipient in any way.

Also, to avoid the problem of immune rejection, scientists are experimenting with different research strategies to generate tissues that will not be rejected.
To summarize, the promise of stem cell therapies is an exciting one, but significant technical hurdles remain that will only be overcome through years of intensive research.

VIII. The Production Of Human Embryonic Stem Cells By Somatic-Cell Nuclear Transfer

The production of human embryonic stem cells by somatic-cell nuclear transfer depends on a profound but obscure event that takes place when the nucleus of a "donor" somatic cell is injected into an enucleated ovum. Somehow, the cytoplasm of the oocyte reprograms the chromosomes of the somatic cell's nucleus so that the newly formed cell becomes pluripotent. The cell develops into a blastocyst, from which embryonic stem cells can be derived that carry a set of chromosomes identical to that of the donor. The "tailored" embryonic stem cells thus derived have fueled hope for new treatments for degenerative diseases such as type 1 diabetes and Parkinson's disease. They are believed to be pluripotent that is, they can differentiate, under appropriate conditions, into cells of any type. With a nuclear complement that is identical to that found in the somatic-cell donor, they are unlikely to be rejected by that donor.

In a recent study, Cowan and colleagues tested the hypothesis that, like the oocyte's cytoplasm, the human embryonic stem cell can also reprogram the chromosomes of a somatic cell. They encouraged the fusion of fibroblasts and embryonic stem cells by coculturing cells of both types in an agent that facilitates membrane fusion, and they obtained stable tetraploid hybrid cells, each of which had a single nucleus. These cells looked and behaved like embryonic stem cells. For example, a protein characteristic of embryonic stem cells was expressed from RNA transcribed from a fibroblast chromosome; the cells seemed to be immortal (they have been passaged more than 50 times). They developed and differentiated into embryoid bodies (in vitro) and teratomas (in vivo) each of these had tissues expressing markers characteristic of each of the three germ-cell layers (endoderm, mesoderm, and ectoderm). Thus, the hypothesis would seem to be correct: human embryonic stem cells can reprogram adult somatic-cell chromosomes after cell fusion. Additional support is provided by similar findings previously obtained with mouse cells.

There is some risk that people who are seeking to place restrictions on research into the biology of human embryonic stem cells may misinterpret these findings, arguing that the new technique represents an alternative approach to the generation of "chromosomally tailored" human embryonic stem cells that have therapeutic potential. Kevin Eggan, one of the investigators in this study, says he is "very disappointed" by this prospect and emphasizes that the study "does not deliver a methodology that can replace human embryonic stem cells." Although this finding will inspire further studies to identify and determine the mechanism of action of the critical factors that reprogram chromosomes, the hybrid cells cannot generate embryonic stem cells and, because they are tetraploid, their therapeutic potential is nil.

Chromatin Decondensation and Nuclear Reprogramming by Nucleoplasmin

Somatic cell nuclear cloning has repeatedly demonstrated striking reversibility of epigenetic regulation of cell differentiation. Upon injection into eggs, the donor nuclei exhibit global chromatin decondensation, which might contribute to reprogramming the nuclei by derepressing dormant genes. Decondensation of sperm chromatin in eggs is explained by the replacement of sperm-specific histone variants with egg-type histones by the egg protein nucleoplasmin (Npm). However, little is known about the mechanisms of chromatin decondensation in somatic nuclei that do not contain condensation-specific histone variants. Here we found that Npm could widely decondense chromatin in undifferentiated mouse cells without overt histone exchanges but with specific epigenetic modifications that are relevant to open chromatin structure. These modifications included nucleus-wide multiple histone H3 phosphorylation, acetylation of Lys 14 in histone H3, and release of heterochromatin proteins HP1ß and TIF1ß from the nuclei. The protein kinase inhibitor staurosporine inhibited chromatin decondensation and these epigenetic modifications with the exception of H3 acetylation, potentially linking these chromatin events. At the functional level, Npm pretreatment of mouse nuclei facilitated activation of four oocyte-specific genes from the nuclei injected into Xenopus laevis oocytes. Future molecular elucidation of chromatin decondensation by Npm will significantly contribute to our understanding of the plasticity of cell differentiation.

REFERRNCES

Data cited in this review were gathered and assembled from the Stem Cell Information Center. The link to this cite is : http://stemcells.nih.gov/info/.